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A Promiscuous Wallflower: The Rare Zayante Sandhills Endemic Erysimum Teretifolium (Brassicaceae) Benefits From Outcrossing

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A PROMISCUOUS WALLFLOWER: THE RARE ZAYANTE SANDHILLS ENDEMIC *ERYSIMUM TERETIFOLIUM* (BRASSICACEAE) BENEFITS FROM OUTCROSSING

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ABSTRACT

A PROMISCUOUS WALLFLOWER: THE RARE ZAYANTE SANDHILLS ENDEMIC, *ERYSIMUM TERETIFOLIUM* (BRASSICACEAE) BENEFITS FROM OUTCROSSING

by Miranda K. Melen

The Zayante sandhills ecosystem of Santa Cruz County, California, contains a rich mosaic of plants and animals adapted to unusual soils and microclimates. In this environment, local adaptation of populations is expected to lead to greater endemism and outbreeding depression. Anthropogenic fragmentation has further isolated populations, however, which paradoxically could result in inbreeding depression. One of these plants, *Erysimum teretifolium* (Ben Lomond wallflower) is an endangered endemic herb in the Brassicaceae family. This study assessed genetic mixing across remnant patches of *E. teretifolium* in order to estimate the effects of fragmentation and the reliance on pollinators. A captive breeding colony was established from seeds collected from four locations across the Zayante sandhills and used in 161 pollinations. Individual allele frequencies were measured using microsatellites, and pollination potential was evaluated through insect camera trapping. Self-pollinations produced 6.5 times fewer seeds than outcrosses. There is no evidence of outbreeding depression. Results suggest that there is a potential for inbreeding depression; however, it is possible that self-incompatibility and reliance on pollinators for outcrosses across geographic divides may be buffering effects. Implications for managers include evidence that geographic origin of individuals may not be critical for reintroduction; however, conservation of the pollinator community should remain a priority.

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INTRODUCTION

Five mass extinctions have been identified from the fossil record (Jablonski and Chaloner 1994; Raup and Sepkoski 1982). Today, in the Holocene epoch, we are currently witnessing global habitat modification, fragmentation, and destruction (Steffen, Crutzen, and McNeill 2007). These monumental changes to the Earth may be propelling us to a sixth mass extinction, commonly described as the Anthropocene extinction, due to the unprecedented rate of species loss from human-caused habitat changes (Steffen, Crutzen, and McNeill 2007; Wake and Vredenburg 2008). Human impact is adversely affecting biodiversity hot spots and, therefore, areas with high endemism and rarity of species are at enormous risk for extinction (McKay et al. 2005).

The central coast of California is considered a hot spot for biodiversity and contains many unique ecosystems (Calsbeek, Thompson, and Richardson 2003; Scott, Standiford, and Pratini 1995). More endemic taxa and subspecies are found in the California Floristic Province than in any other part of North America of comparable size (Calsbeek, Thompson, and Richardson 2003; Davis et al. 2007). Many of these vulnerable ecosystems are experiencing land-use change. Among these ecosystems is the Santa Cruz Mountains, now threatened by urbanization, destructive forms of recreation, and sand mining (Hill and O'Malley 2010; McGraw 2004b). Small plant populations found in geographically fragmented habitats are more at risk of extinction due to genetic drift, limited mate selection, and increases in inbreeding (Honnay and Jacquemyn 2007).

Island Biogeography

Island biogeography theory predicts that geographic distance plays a major role in molding insular diversity (MacArthur and Wilson 1963). Large islands near the mainland tend to have a greater number of species than small islands that are near the mainland. This theory also predicts that large islands that are near the mainland will have a higher number of species and a lower extinction rate than large islands that are far away from the mainland. Developed for ocean islands and species diversity, this theory can also be used for conservation on land and applied to genetic diversity where species restricted to continental island-like habitats should be governed by the geography of "patch dynamics" (Barbará et al. 2008; Edmands 2007; Pickett 1985).

When organisms are dispersal limited, geographic distance may reflect genetic distance among populations (Levin 1981; Levin 1984). With increasing genetic isolation, local adaptation to different habitats can arise (Rice and Emery 2003; Wright, Stanton, and Scherson 2006). Different forms of fragmentation can lead to extreme cases of either inbreeding depression or outbreeding depression with opposite management implications. Therefore, it is critical for land managers to consider these effects before implementing restoration projects (McKay et al. 2005).

Fragmentation in Evolutionary Time vs. Anthropogenic Fragmentation

Occurring over the evolutionary time scale, geomorphological features can create habitat barriers that fragment environments (Aguilar et al. 2008; Dias et al. 2013). Habitats that have been fragmented on evolutionary time scales can have high numbers of

endemic species and increased species richness because increased exposure to natural fragmentation can drive speciation (Quinn and Harrison 1988). Mountaintop species are restricted by climate and range of habitat, and in California, Nevada, and Utah, vast deserts separate these ecosystems, causing an island-like pattern for biodiversity (Brown 1971). Other examples of isolated continental habitat include lake fauna that are surrounded by dry land, which inherently disrupts the movement of aquatic life from one lake to another, or fragmented forests and woodlands (Barbour and Brown 1974; Grashof-Bokdam and Geertsema 1998; Holycross and Douglas 2007). Small isolated populations may have low genetic diversity and can be catalysts for speciation, such as biodiversity hotspots, or detrimental to resilience against environmental fluctuation.

Geomorphological features separating populations of once connected species can affect the genetic distinctiveness of a population if it becomes locally adapted to a region (Dias et al. 2013; Holycross and Douglas 2007; Wright, Stanton, and Scherson 2006). Local adaptation is beneficial for selecting genes that are most fit for environmental conditions. Fitness is a relative measure of reproductive success of an organism in passing genes from one generation to the next. Environmental conditions select for different genes to be most fit. The simplest measure of fitness can be the number of offspring, but in the long run, fitness is a measure of how many offspring survive to reproduce. For plants, fitness is best measured across a range of life history stages from seed to germination, seedling survival, growth, and reproduction.

Natural fragmentation can also decrease a population's genetic diversity and, in extreme cases, lead to outbreeding depression. Outbreeding depression occurs when

locally adapted species mate with individuals from another location (Edmands 2007). This dilution of the genetic pool can cause detrimental problems for the local population by promoting non-specific genes rather than localized fit genes.

Human disturbance is producing an unprecedented change in land use and negatively impacting biodiversity by causing previously connected habitat ranges to become disjunct (McKay et al. 2005). As urbanization, recreation, and resource mining continue to fragment and isolate habitats, the potential for inbreeding greatly increases. Fragmented habitats expose small plant populations to higher chances of extinction due to loss of genetic variability and increased levels of self-fertilization, which can lead to inbreeding (Aguilar et al. 2006; Honnay and Jacquemyn 2007; Saunders, Hobbs, and Margules 1991). Inbreeding can decrease fitness and reduce genetic diversity, resulting in inbreeding depression (Barrett 2002; D. Charlesworth and B. Charlesworth 1987; Edmands 2007; Richter, Crother, and Broughton 2009). Inbreeding depression is assumed when increasing relatedness of parents gradually reduces the fitness of the species. For small isolated plant populations, inbreeding can be catastrophic for reproduction.

Population genetics, population structure, demography, and environmental variables affect mating systems, leading plants to exhibit a range of reproductive mechanisms including autogamy (self-fertilization) and outcrossing (cross-fertilization) (Steets et al. 2007). These reproduction strategies are driven by both evolutionary and environmental factors (Steets et al. 2007). Therefore, understanding plant mating

systems is critical for restoration practitioners to successfully conserve rare or endangered small populations (McKay et al. 2005).

Plant Mating Systems

Self-fertilization (self-pollination) occurs when pollen is transferred to the stigma of the same flower within the same plant, or to the stigma of a different flower on the same plant. About 10 to 15% of plants rely on self-pollination for reproduction (Goodwillie, Kalisz, and Eckert 2005; Wright, Kalisz, and Slotte 2013). Self-pollination selects for localized genes and can lead to increased rates of speciation and adaptation. This mating system is beneficial when pollinators are limited, plant species are widely dispersed over their geographical range, and the habitat is relatively stable with a predictable microclimate (Busch 2005; Eckert and Schaefer 1998).

Baker's Law states that in fragmented habitats where mate selection is low, self-compatible mating systems can arise because a single plant can colonize an isolated area (Pannell and Barrett 1998). Eckert and Schaefer (1998) found that self-compatible species tend to live in marginal, but stable, habitats. Self-pollination is advantageous in environments requiring adaptations that occur through the rise of a beneficial genotype, because it effectively increases probabilities that a locally adapted genotype will pass to the next generation. A disadvantage of self-pollination is a resulting smaller gene pool. This can lead to a poor ability to react to environmental stress and therefore make a species more at risk of extinction (Barrett 2002; D. Charlesworth and B. Charlesworth 1987; Edmands 2007; Willi, Van Buskirk, and Hoffmann 2006).

Self-incompatibility is a mechanism that plants use to protect themselves from inbreeding depression by implementing a mating system that only accepts pollen from genetically dissimilar plants (D. Charlesworth and B. Charlesworth 1987; Guo et al. 2009; Kao and McCubbin 1996; Mena-Ali, Keser, and Stephenson 2008). This function is activated during either the initial placement of the pollen on the stigma or during the growth of the pollen tubes. Busch (2005) notes that self-compatible mating systems are often found in island-like habitats due to limited access to mates and degraded environmental conditions. Anderson and Busch (2006) found that when pollinator-mediated selection is relaxed in *Leavenworthia* (Brassicaceae), the traits associated with the movement of pollen are modified, causing an evolutionary change in the shape and size of floral organs. Self-compatible *Leavenworthia* taxa had high rates of self-pollination resulting in weak stamen-petal and pistil-petal length correlations, whereas self-incompatible species had stronger correlations (Anderson and Busch 2006).

Outcrossing, the exchange of genetic material between two unrelated individuals, allows plants to maintain diverse gene pools, increase fitness, and reduce the level of disease and genetic abnormalities that can result from inbreeding (Barrett 2002; D. Charlesworth and B. Charlesworth 1987; Edmands 2007). Plants that rely on outcrossing implement mechanisms to prevent or inhibit unwanted pollen donations from the same flower (Barrett 2003; Guo et al. 2009). These plants use either spatial or temporal separation of sexual structures to limit the transfer of pollen to the pistil of the same flower (Barrett 2002), or through self-incompatibility mechanisms (Anderson and Busch 2006; Busch 2005; Guo et al. 2009).

The physical locations of the pistil versus the anthers limit the risk of self-fertilization with varying costs to the success of precise pollen transferal (Barrett 2002). Populations with single floral phenotypes have a high level of precision for each pollen transfer using animal pollinators; however, the level of interference with unwanted self-pollination can be detrimental. Herkogamy places the stigma and anthers at different vertical locations within the flower to prevent a pollinator from transferring pollen within the same flower. This leads to lower rates of successful pollination due to the placement of pollen on the pollinator and how it matches up with the next stigma it encounters (Barrett 2002). It is also possible for species to have more than one flower morph in a population to regulate the transferal of genetic material. This ensures no sexual interference and guarantees that pollen is precisely applied when a pollinator moves from the first morph to the second. This approach can be costly in small populations or in areas where individuals are widely dispersed, as a pollinator must visit an opposite morph in order for a successful pollination to occur.

Pollination Theory

Sixty to 70% of flowering plants rely on insect pollinators for reproduction (Potts et al. 2003). For flowering plants that rely on insect-pollination, the movement of pollen is affected by population size, spatial distribution of plants, behavior of pollinators, and floral characteristics (Barrett 2003; Cresswell et al. 1995; Mayer, Van Rossum, and Jacquemart 2012; Young, Dunning, and von Hasseln 2007). Mayer, Van Rossum, and Jacquemart (2012) note that insect visitation rates often increase in populations that are

larger or have higher flower densities. Specialized pollinators are vital to the survival of some plants that have coevolved with specific insects to ensure proper pollination (Bawa 1990; Packer et al. 2005). Ultimately, small plant populations have fewer mates available and offer less reward for insect pollinators. With fewer pollinators, visits per flower decline, limiting pollen transfer within the population and leading to poor seed production (Campbell and Husband 2007).

Insects are attracted to plants producing showy flowers, alluring scents, vivid colors, and plentiful nectar rewards and pollen sources (Bauer 1983; Kremen et al. 2002; Kremen et al. 2004; Welzel 2011; Winfree et al. 2007). Flower morphology often dictates which insects will pollinate; however, pollinators do not necessarily adhere to pollination syndromes (Bauer 1983; Bosch and Waser 2001; Galen and Newport 1987; Waser et al. 1996). Waser et al. (1996) describe how the pollinator's lifespan and required travel distance affect the foraging habits of the insect. Fenster et al. (2004) note that pollinating insects seek out flowers with high rewards of nectar and pollen, and therefore will frequent those species or individual plants that have larger pollen rewards and faster nectar refill rates.

Fragmented or degraded landscapes compromise the health of insect pollinators and impact plants by reducing rates of outcrossing and increasing the potential for inbreeding (Broadhurst 2008; Eckert et al. 2009; Mayer, Van Rossum, and Jacquemart 2012). Research on correlations between patterns of pollen dispersal and levels of habitat degradation is important for restoration management plans (Mayer, Van Rossum, and Jacquemart 2012). Managers for geographically isolated habitats need to understand

whether fragmentation on evolutionary time scales or anthropogenic fragmentation is impacting species, and whether pollinators can act as genetic corridors for outcrossing plant species.

STUDY SYSTEM

Zayante Sandhills

The Zayante sandhills (sandhills) are located in the Santa Cruz Mountains in central coastal California, USA, approximately 120 km south of San Francisco and about 18 km east of the Pacific Ocean. The marine influence of the Pacific Ocean and the obstruction of interior wind patterns from the Santa Cruz Mountains create a mild, Mediterranean climate with cool, wet winters and hot, dry summers (U.S. Department of Agriculture 1980). Annual rainfall is between 100 and 140 cm with average temperatures ranging from about 14 to 35° C (McGraw 2004a). The land is an important resource for Santa Cruz County; limestone and shale are mined for cement production, and sand and gravel deposits are quarried for glass and other construction material (U.S. Department of Agriculture 1980).

The sandhills are located at elevations of about 75 to 460 m above sea level. Soils are poorly developed and composed of excessively drained coarse sand, found on slopes of 5 to 50%, with low levels of nutrients (McGraw and Levin 1998; McGraw 2004b; U.S. Department of Agriculture 1980). This unusual edaphic condition for the elevation resulted from the weathering of the Santa Margarita formation, an ancient seabed that was uplifted and exposed during the formation of the Santa Cruz Mountains during the Miocene era (McGraw 2004b; U.S. Department of Agriculture 1980). Naturally fragmented, the sandhills are isolated patches of xeric soils surrounded by intervening mesic redwood forests and mixed evergreen forests (Figure 1). Due to the unique soil

quality, climatic conditions, and relative isolation, plant and animal communities exhibit high rates of endemism (McGraw 2004b).

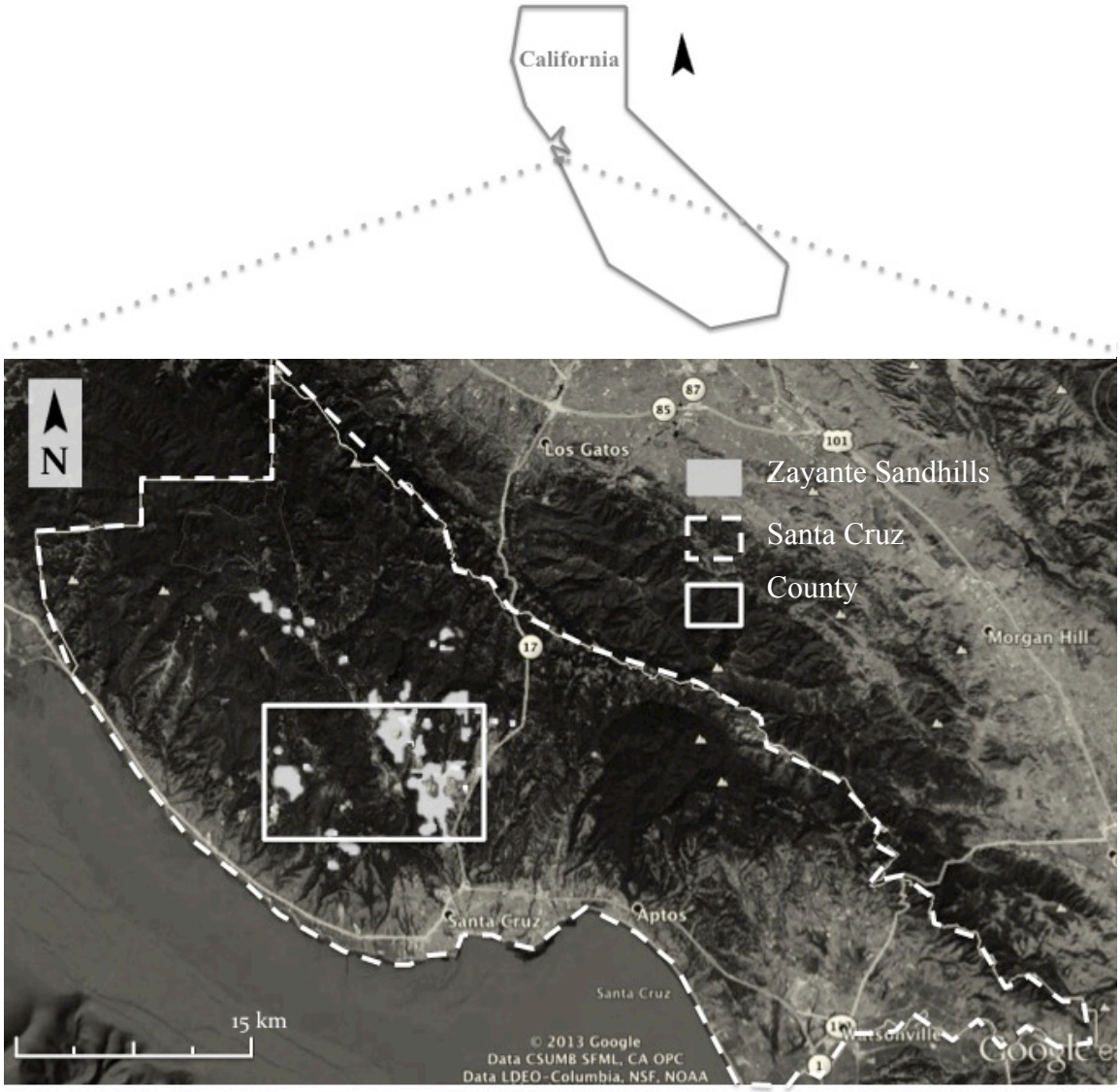

Figure 1. Zayante sandhills map. The natural fragmentation of the sandhills is evident in the patches of xeric soils that are isolated by intervening mesic redwood forests and mixed evergreen forests. Image source: Google Earth™ 2013.

Covering about 3% of Santa Cruz County, the sandhills ecosystem consists of two sand-based habitats: sand chaparral and sand parkland (Granite Rock Company 1998;

Hill and O'Malley 2010; McGraw 2004b). The dominant sandhills habitat is sand chaparral, which is identified by large manzanita shrubs such as *Arctostaphylos silvicola* (silverleaf or Bonny Doon manzanita) and many tree species, such as *Pinus attenuata* (knobcone pine), *Pinus ponderosa* (ponderosa pine), and *Quercus agrifolia* (coast live oak) (McGraw 2004b). Sand parkland is predominantly found on exposed ridges with little tree canopy, except for occasional *P. ponderosa*. These steep hills are host to a diverse collection of plant species, many of which are rare or endangered. The U.S. Fish and Wildlife Service (1998) estimates that sand parkland once covered up to 240 ha; however, due to land-use change, fewer than 80 ha remain.

Human activities, such as urban development, recreation, and mining, have led to extreme habitat loss in the sandhills (Hill and O'Malley 2010; McGraw 2004b). The region between California State Route 9 and California State Route 17 has seen increased urbanization in addition to recreational use. Historically, there were many sand and gravel mines, only a few of which are still in operation. However, mining practices led to extreme land degradation where soils were removed for manufacturing, severely impacting local habitat communities.

Habitat loss affects a suite of species adapted to the sandhills. Three endangered plants found in the sandhills are federally listed: *Erysimum teretifolium* (Ben Lomond wallflower), *Chorizanthe pungens* var. *hartwegiana* (Ben Lomond spineflower), and *Cupressus abramsiana* (Santa Cruz cypress) (McGraw and Levin 1998; Murphy 2003; U.S. Fish and Wildlife Service 1997). Two endangered insects are endemic to the sandhills, the *Trimerotropis infantilis* (Zayante band-winged grasshopper) and the

Polyphylla barbata (Mount Hermon June beetle). Preferring open sand parkland regions, *T. infantilis* predominantly relies on *Lupinus albifrons* var. *albifrons* (silver bush lupine) for forage (Chu 2002). Sand parkland is also the habitat for *P. barbata*, a generalist feeder with remnants of angiosperms and fungi in its frass (Hill and O'Malley 2010). There are many undescribed plant and insect species, sandhills subspecies, ecotypes, disjunct populations, and species of concern (e.g., *Dipodomys venustus venustus*, the Santa Cruz kangaroo rat).

The patchy and isolated distribution of these sandhills provides an excellent opportunity to examine the roles of geographic distance and genetic divergence in mating success in a continental island-like habitat. However, with anthropogenic fragmentation compounding naturally isolated habitat, it is unclear how reproductive success affects native and endemic plant species in the sandhills. One of the endangered species, *E. teretifolium*, is endemic to sand parkland and provides an excellent case study of small population response to extreme fragmentation.

***Erysimum teretifolium* (Ben Lomond wallflower)**

A member of the Brassicaceae family, *E. teretifolium* is a monocarpic, biennial herb found in the sandhills (McGraw 2004b). The leaves have purple-green, linear blades, finely toothed with terete margins, and are identifiable by their double-barbed leaf hairs. Leaves form a basal rosette during the vegetative stage, whereas older leaves senesce and expose a bumpy, almost woody, stem that can extend 5 to 10 cm above ground. As the plant reaches maturity, generally in the second or third growing season,

the basal rosette produces a cluster of undeveloped buds, which then bolts, forming an inflorescence about 2.5 to 8 dm (Jepson Floral Project 2013). Flowers are produced on racemose inflorescences with each flower having four petals, six stamens, and developing long, slender fruits called siliques with two deciduous valves. An inflorescence may have five to 10 open flowers at one time in various stages of pollen viability. Flowers are bright yellow and offer a faint, sweet-cream scent. If the main bud cluster is removed by local herbivores, such as *Odocoileus hemionus columbianus* (black-tailed deer), *E. teretifolium* may develop axillary inflorescences to compensate or even overcompensate.

In a survey of the sandhills, the U.S. Fish and Wildlife Service (1998) estimated that about 16 populations remain. By 2011, only six were known, six others were said to be located on private land, and were small in number and unreviewed (Parker, Weitz, and Webster 2011). Other plants found in association with *E. teretifolium* are *Acmispon glaber* var. *glaber* (deerweed), *Monardella undulata* ssp. *undulata* (curly-leaved coyote mint), *Eschscholzia californica* (California poppy), *Mimulus androsaceus* (rockjasmine monkeyflower), *Bromus tectorum* (cheat grass), *L. albifrons* var. *albifrons* (silver bush lupine), and a disjunct variety of *P. ponderosa* (ponderosa pine).

Land managers interested in conserving *E. teretifolium* should consider the overall effects of island biogeography on this plant, and in particular exactly how much genetic mixing is required to keep the populations viable, or to reintroduce them to extirpated populations. Either inbreeding depression or outbreeding depression can be problematic when only small fragmented populations remain. Therefore, managers need to understand the mating system of plants in order to determine if they successfully self-

fertilize, or if they depend on pollinators to outcross, as well as to assess how geography and habitat barriers impact genetic divergence and fitness.

RESEARCH QUESTIONS

RQ1: Is *E. teretifolium* self-compatible?

RQ2a: Does linear geographic distance between parents affect fitness or reflect genetic divergence?

RQ2b: Does nonlinear geographic distance between parents affect fitness?

RQ2c: Does genetic divergence between parents affect fitness?

RQ2d: Does the population source affect fitness?

RQ3: Who are some of the primary insect pollinators, and what is the frequency of visitation?

METHODS

Study Design

Using seeds collected from four wild populations, a captive breeding population was established in order to complete this study (Figure 2). The four *E. teretifolium* populations used for this study were Quail Hollow Ranch County Park (Quail Hollow Park), San Lorenzo Valley Water District Olympia Wellfields (Water District), Randall Morgan Sandhills Preserve (Morgan Preserve), and Bonny Doon Ecological Reserve (Eco Reserve) (Figure 3). From the captive breeding population, the plant mating system was evaluated in a greenhouse setting by conducting 161 manual pollinations on 50 plants over two flowering seasons, 2012 and 2013. The linear geographic distance between individual plant crosses in the captive breeding population was determined by using a GPS data logger to determine the seed origin location at each wild population site. Leaf DNA samples were removed from each plant in the captive breeding population to identify genetic divergence between crosses. In wild populations, pollinators attracted to *E. teretifolium* were surveyed with video cameras to determine the visitation rate of insect pollinators to reproductive floral parts.

Background

In 2010, seeds were harvested from Quail Hollow Park, Water District, Morgan Preserve, and Eco Reserve by Parker from the University of California at Santa Cruz (UCSC). Three patches at each location were selected for seed harvesting, except Eco Reserve where only two patches were harvested due to the smaller number of patches

available. The distance between patches varied from 10 to 162 m. At the UCSC greenhouse, seeds were germinated in four-inch pots in August 2010. Germination was scored, plants were thinned to one per pot, placed in a randomized order from September 4 through 6, and allowed to grow.

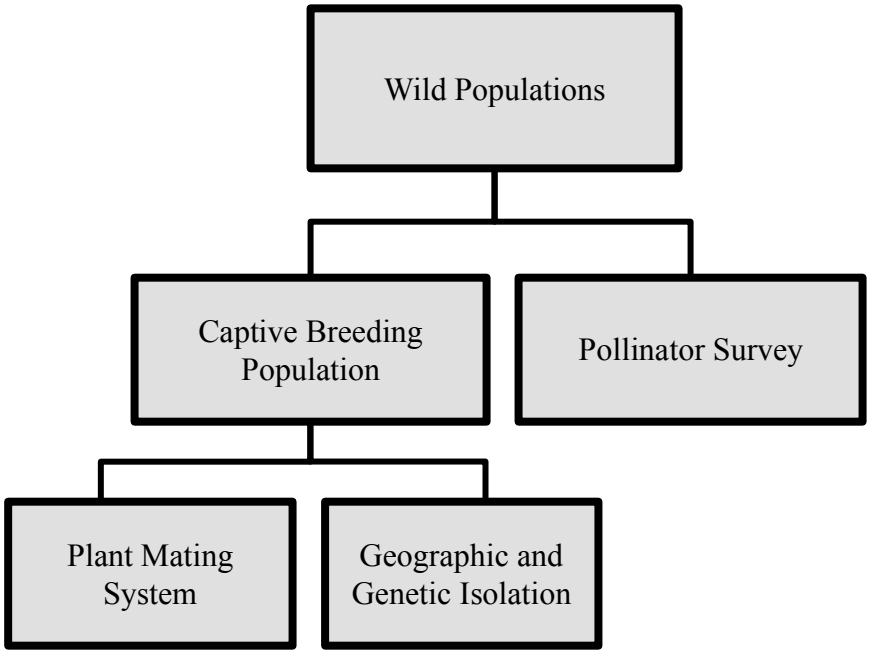

Figure 2. Research overview. A captive breeding population was established from four wild populations to study the mating system of *E. teretifolium* and the effects of geographic and genetic isolation. A spring diurnal pollinator survey was conducted at two wild population sites.

On March 27 and 28 of 2012, all plants were transplanted to one-gallon pots and transported to the Santa Clara University (SCU) Biology Outdoor Experimental Garden (Quail Hollow Park $n = 76$; Water District $n = 75$; Morgan Preserve $n = 76$; Eco Reserve $n = 46$). Plants were kept outside for six weeks to experience some late winter dormancy. In May, plants were moved to an SCU greenhouse where they flowered from June

through August. During this time, 14 plants flowered (Quail Hollow Park *n* = 1; Water District *n* = 4; Morgan Preserve *n* = 8; Eco Reserve *n* = 1) and were hand-pollinated (self-pollination *n* = 14; outcross pollination *n* = 31). Seeds were harvested two to three months after initial pollination (August 28 – December 12), and the plants were moved back outside to the Outdoor Experimental Garden. On March 21, 2013 all plants were moved back into the SCU greenhouse, and 36 plants (Quail Hollow Park *n* = 11; Water District *n* = 8; Morgan Preserve *n* = 8; Eco Reserve *n* = 9) were selected for pollination (self-pollination *n* = 29; outcross pollination *n* = 87).

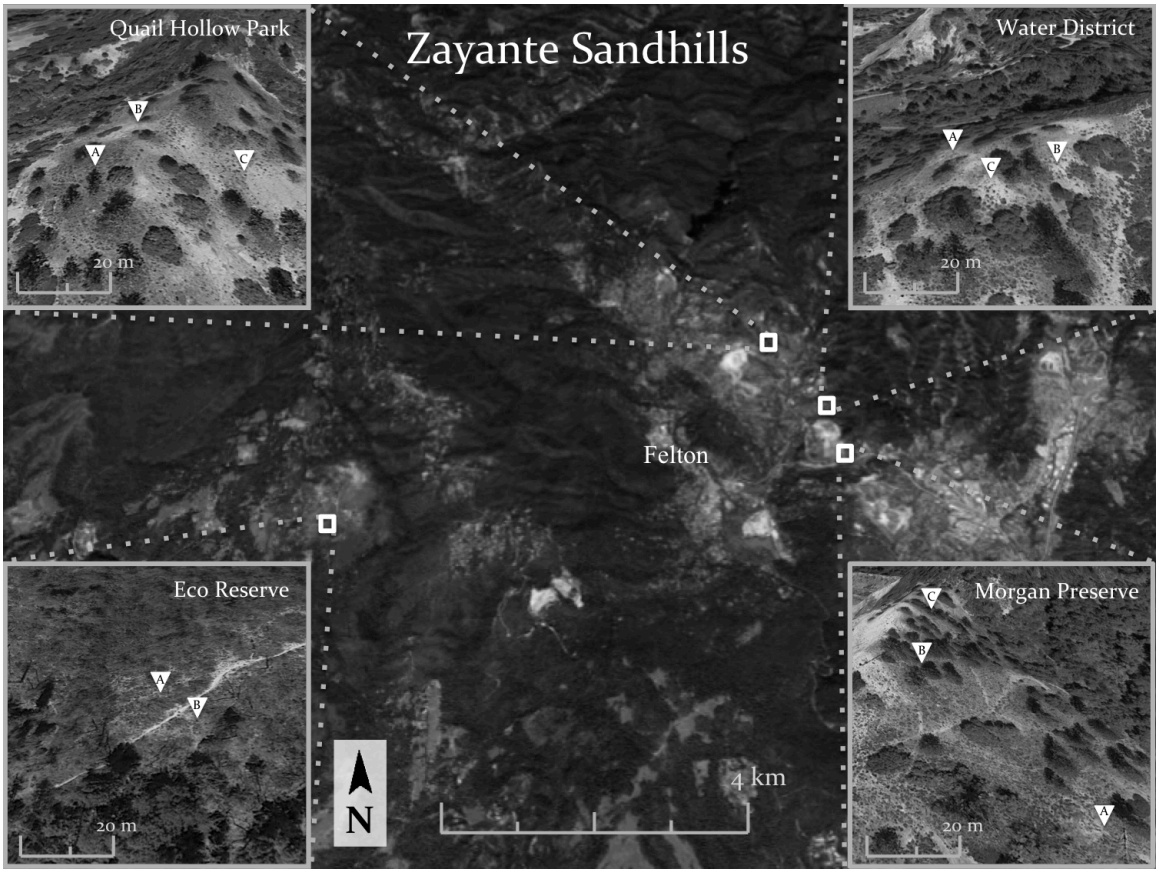

Figure 3. Sandhills study sites. The four *E. teretifolium* population sites used for this study were Quail Hollow Park, Water District, Morgan Preserve, and Eco Reserve. Image source: Google Earth™ 2013.

Plant Mating System

In order to assess whether *E. teretifolium* is self-compatible, 161 crosses were conducted (self-pollination $n = 43$; outcross pollination crosses $n = 118$). Each population source was represented: 12 from Quail Hollow Park, 12 from Water District, 16 from Morgan Preserve, and 10 from Eco Reserve. All pollination types were attempted on each plant in order to control for maternal effects.

Light and Temperature

While at UCSC, seedlings were exposed to supplemental light for 12 hours during the day and 12 hours at night using 100-watt grow lights. Plants were watered by hand one to two times per week, and fertilized with 100 ppm of Peter's 20:20:20 (NPK) every two to three months. At SCU, greenhouse temperatures were kept below 29.4° C, and natural sunlight was supplemented with the same lighting schedule used at UCSC, implementing 400-watt Hortilux® lights. When wilting was detected, plants were watered for 5 sec each on the "gentle" setting using a Dramm™ spray nozzle model 80-12704.

Insect Control

Insect control methods included sticky traps, manual removal, and spray pesticides. To target flying insects, such as thrips, Blue & Yellow Card Traps by Alpha Scents, Inc.™, were hung in the greenhouse. Plants were visually inspected before

watering and insects (e.g., aphids) were manually pinched off the leaves and stems of infected plants. A steady stream of water was used to reach insects that were difficult to access. Whenever insects threatened the health of the plants, aphids were controlled using Safer® Brand Insect Killing Soap and Bonide Captain Jack's Deadbug Brew® was used on thrips.

Bud Preparation

Pollination treatment types were randomized for each flowering plant using the RAND and RANK function in Microsoft® Excel 2011 v.12.3.6, and each treatment type was assigned a unique paint color. In order to ensure control over the pollination process, each bud was emasculated prior to pollination to remove male reproductive parts, leaving only the female part. Emasculations were performed on all five pollination treatment types using the first 10 buds to develop on each plant. Buds were selected from the outer perimeter of the bud cluster. Using curved forceps, sepals and immature petals were peeled back to expose the anthers and pistil. Straight forceps were used to pluck each of the six anthers near the base of the filament and completely remove them from the flower. The sepals were then moved back into place to protect the flower interior, and the pedicel of the flower was marked with the assigned treatment paint color.

Pollination Treatment

Pollination occurred when the majority of *E. teretifolium* plants were in full bloom during 2012 and 2013. After emasculation, randomized crosses were performed.

One treatment was an emasculation control to show that the act of emasculation did not produce seeds, that there were no airborne pollen in the greenhouse, and that the greenhouse was pollinator free. The other treatments were self-pollination, and then varying degrees of outcross pollinations based on geographic distance to the mother plant. Pollination treatments were randomized and pre-assigned to buds on flowering plants. All pollination types were attempted on each plant in order to control for maternal effects. Buds were emasculated on the first day of treatment and then received pollen from the same donor on days four, five, and six. Pollen was applied to the surface of the stigma using a flat toothpick. A dry stigma receptivity test using Peroxtesmo KO by Macherey-Nagel found that the stigma was receptive from days one to nine of treatment (Quail Hollow Park $n = 6$; Water District $n = 6$; Morgan Preserve $n = 8$; Eco Reserve $n = 7$), and therefore was receptive during pollen donations on days four, five, and six.

Seed Harvesting

Once pollinated, flowers took about two months to set seed, at which point the siliques were harvested for analysis. Siliques were removed from the plant and placed in pre-labeled paper envelopes. The length of each silique, number of seeds, and average weight of the seeds were recorded for each cross within 42 days of harvesting.

Geographic and Genetic Isolation

Linear Geographic Distance Between Parents

Seeds were originally collected from a total of 14 sites (representing four populations) in 2010. Sites were reviewed in 2013 to determine distances for each cross completed in the mating system experiment. GPS points were logged with a Garmin® Oregon 550t, plotted with Google Earth™ 2013, and distances were estimated with Google Earth™ 2013 and Microsoft® Excel 2011 v.12.3.6. Crosses between geographically adjacent plants were estimated at 5 m due to lack of precise locality data. Geographic distances between parents of the outcrossed individuals ranged from 5 to 8,238 m (crosses involving the disjunct Eco Reserve population). Self-pollinations were given values of zero for geographic distances. The average geographic distance between parent plants was 579 m, and the median was 1,368 m.

Nonlinear Geographic Distance (Habitat Barriers) Between Parents

Geographic connectivity is not necessarily defined in linear terms. In many cases, geographic distance can be based on habitat barriers that prevent a species from easily moving from one part of its range to another. Nonlinear habitat barriers in the Santa Cruz Mountains include elevation and topography, patches of mixed evergreen and redwood forests, and human land uses including urbanization and mining. For plants and animals that are restricted to specific environmental conditions, such as soil, light availability,

moisture and other variables, such habitat barriers can isolate and prevent populations from exchanging genetic material even over relatively modest linear distances.

In order to evaluate whether such habitat barriers may play a role in *E. teretifolium* fitness, parental crosses were categorized into three broad levels of geographic distance: self-pollination, "within-population", and "between-population" (Figure 4). Self-pollinations were those used in the plant mating system experiment described above; crosses between two parent plants from seed collected less than 50m apart and across no significant identifiable geographic habitat barriers were considered "within-population," whereas crosses between two parent plants spanning an identifiable geographic divide were labeled "between-population" (Figure 5).

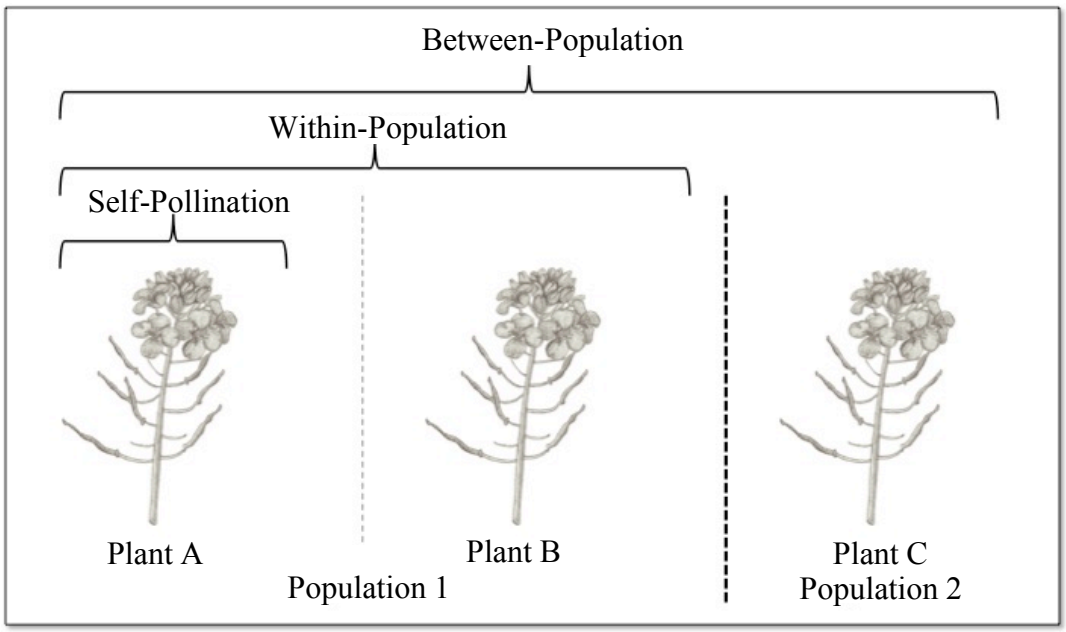

Figure 4. Nonlinear geographic distance (habitat barriers). Three pollination cross types were used to compare the seed production from self-pollination, within-population, and between-population.

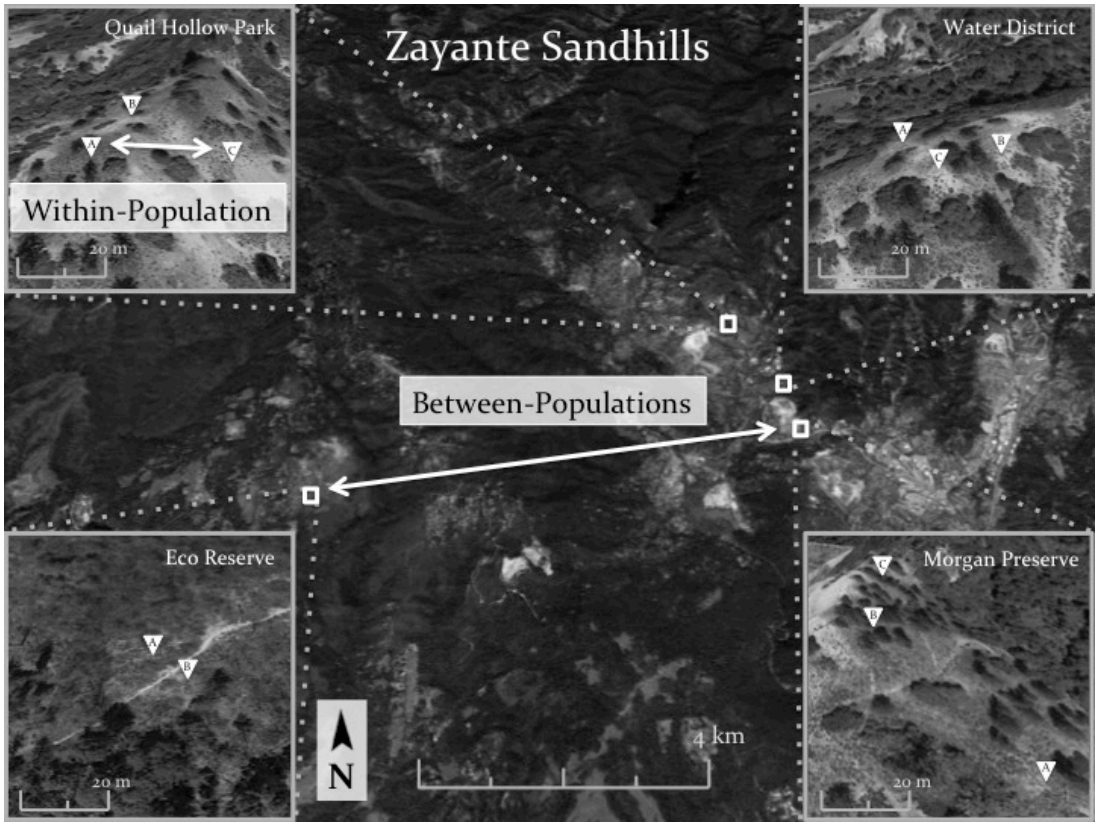

Figure 5. Sandhills nonlinear geographic distance (habitat barriers). Within-population and between-population cross types. Image source: Google Earth™ 2013.

Genetic Divergence Between Parents

In April 2012, two new leaves and two mature leaves were collected from 277 plants in the captive breeding population, placed in a coin envelope, labeled with the plant ID, and stored at -20° C for later DNA extraction. From June through August 2012, *E. teretifolium* leaf DNA was extracted at SCU using a Macherey-Nagel kit and then amplified using microsatellites (Herman and Whittall, forthcoming). Microsatellites were separated at Cornell University's Genome Center on an ABI 3730 DNA Sequencer and alleles were scored with Peak Scanner™ Software v1.0 (Thermo Fisher Scientific,

Waltham, Inc., Massachusetts). Four heterospecific microsatellite loci (Muñoz-Pajares et al. 2011), developed for *Erysimum mediohispanicum*, were used to estimate genetic divergence of the parents in each cross. Twenty-four variable bands were identified among the individuals used in this crossing experiment (Herman and Whittall, forthcoming).

The traditional genetic distance approach was not suitable for *E. teretifolium* because of the difficulty assessing heterozygosity in this polyploid. Since the plant was found to be a hexaploid (Herman and Whittall, forthcoming; Price 1987) and exhibits one to six bands per locus, a genetic divergence metric was developed to reflect the number of matched and mismatched alleles corrected by the frequency of each allele from a larger population survey (Herman and Whittall, forthcoming; Equation 1).

$$GD = \frac{\sum_{i=1}^{n_0} d_i}{n}$$

$$\left(Z_i(A) \equiv 0\right) \cap \left(Z_i(B) \equiv 0\right) \rightarrow d = 0$$

$$\left(Z_i(A) \equiv 1\right) \cup \left(Z_i(B) \equiv 1\right) \rightarrow d = f_i$$

$$\left(Z_i(A) \equiv 1\right) \cap \left(Z_i(B) \equiv 1\right) \rightarrow d = f_i - 1$$

Equation 1. Genetic divergence metric. Smaller genetic divergence numbers denoted parent combinations that were more genetically similar, whereas larger genetic divergence numbers indicated parent combinations that were more genetically divergent.

The larger survey took samples from eight populations of *E. teretifolium*, including the four captive breeding populations, and looked at four loci for presence and

absence of alleles. The genetic divergence metric was weighted for individuals with rare alleles and therefore highlighted divergence between individuals. In this equation, n_0 is the total number of markers and n is the number of markers where at least one sample has the allele. The variable d is defined as the marker score for allele i, where f is the frequency of allele i in the population, and $Z_i(A)$ is the binary presence/absence of allele i in individual A.

Population Source

In addition to the potential for geographic distance and genetic divergence to contribute to plant fitness, abiotic factors may differ at each population location and affect fitness. For each cross performed in the mating system experiment, the source populations of the parents were recorded. The average number of seeds produced per silique was compared with the population source of each mother plant to determine if there was a population influence on fitness. Self-pollinations were removed from this comparison because there was an unequal amount among the populations and they rarely produced seeds.

Pollinator Survey

Digital video cameras were used to document the common diurnal pollinators for *E. teretifolium* and the frequency of insect visitations. In Spring 2013, two populations were surveyed: Quail Hollow Park (3/27/13) and Water District (3/29/13). Canon FS22s automatic video cameras were set up on tripods and placed approximately 50 cm from

inflorescences at an angle ranging between 0° (horizontal) and 30° below horizontal. To record flower hours of insect visitations, cameras were focused on inflorescences with five to 14 open flowers (mean = 7.8 flowers/camcorder). Cameras started recording in the field by 7 a.m. and were removed at approximately 7 p.m. allowing for maximum exposure to daytime temperatures that are amenable to diurnal insect movement. Memory cards and batteries were swapped when needed in order to maximize the recording time in the field each day.

DATA ANALYSIS

Plant Mating System

Self-pollinations and outcrosses were compared to determine whether *E. teretifolium* is self-compatible. Using Levene's test, variance was determined to be unequal, and therefore a Welch's t-test in Microsoft® Excel 2011 v.12.3.6 was used. The average number of seeds produced per silique was compared between cross types.

Geographic and Genetic Isolation

Linear Geographic Distance Between Parents

Fitness

In order to assess whether linear geographic distance between parents affects fitness, the average number of seeds produced per silique was regressed on log transformed geographic distance between each cross using StatPlus®:mac LE. Geographic distance was log transformed to correct the leptokurtic distribution of the data (positive excess kurtosis).

Genetic Divergence

Isolation by distance was assessed by regressing genetic divergence on the log transformed geographic distance using StatPlus®:mac LE.

Nonlinear Geographic Distance (Habitat Barriers) Between Parents

In order to assess the effects of geographic habitat barriers on seed set, linear geographic distances were initially categorized into three groups: self-pollination, within-population, and between-population (Figure 5). Seed count data were tested for normality using a Kolmogorov-Smirnov test. As the distribution was found to be non-normal, the average number of seeds produced per silique for different barrier groups was contrasted using a non-parametric Kruskal-Wallis test with a post hoc Dwass-Steel-Chritchlow-Fligner test in SYSTAT 13™ for all pairwise comparisons.

Genetic Divergence Between Parents

Using StatPlus®:mac LE, genetic divergence was regressed on the average number of seeds produced per silique to look for any relationship between parental genetic divergence and seed set outcomes.

Population Source

A Kolmogorov-Smirnov test was used to test for normality of the seed count data and was found to be non-normal in distribution. Therefore, the average number of seeds produced per silique was analyzed using a non-parametric Kruskal-Wallis test with a post hoc Dwass-Steel-Chritchlow-Fligner test in SYSTAT 13™ for all pairwise comparisons to test differences among the average number of seeds produced per silique by source population.

Pollinator Survey

In pollinator observation studies, a common metric is visits per flower hour (VFH) (Bradshaw and Schemske 2003; Chittka and Schürkens 2001; Price et al. 2005). Data were imported from each camera using iMovie™ 11 9.0.9, exported as a .mov file, and played in QuickTime® Player 10.2. The start and stop times were recorded in Microsoft® Excel 2011 v.12.3.6 for each insect that made contact with the reproductive parts of *E. teretifolium*. The number of flower hours was calculated by multiplying the number of video hours by the number of viewable open flowers, thus arriving at 151 flower hours. To determine the average number of visits per flower hour, the number of insect visits was summed and divided by the total number of flower hours. Screen shots were taken for insect order identification.

$$VFH = \frac{\text{\# Insect Visits}}{\text{Flower Hours}}$$

Equation 2. Visits per flower hour.

RESULTS

Plant Mating System

Self-pollinations (n = 43) produced 6.5 times fewer seeds than outcross treatments (n = 118) (t-test, t_{160} = -2.991, P < 0.0001; Figure 6). Self-pollinations that produced seeds had no discernable pattern to correlate to a specific population source. All emasculation controls on plants produced zero seeds, indicating the greenhouse was pollinator free and that emasculations did not result in fertilization.

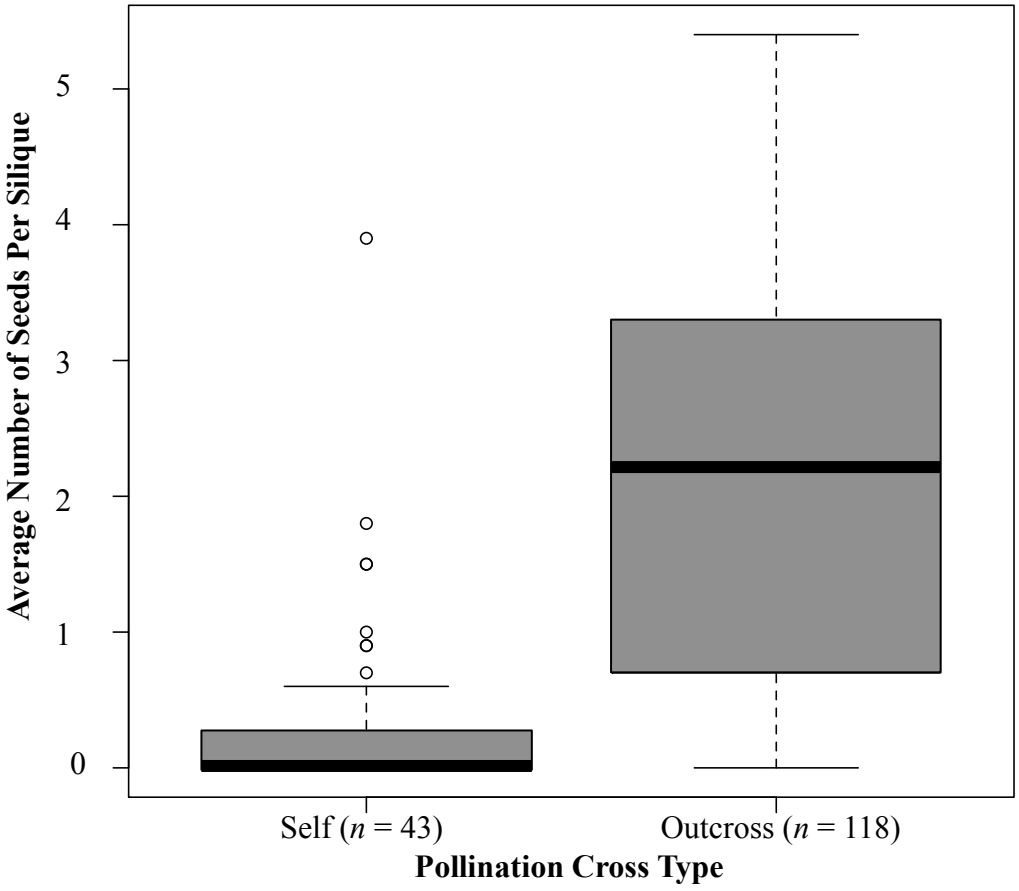

Figure 6. Plant mating system. Self-pollinations (n = 43) produced 6.5 times fewer seeds than outcross treatments (n = 118) (t-test, t_{160} = -2.991, P < 0.0001). A Tukey boxplot showing the median within the interquartile (IQR) range and whiskers of 1.5 IQR. Outliers are represented as open dots.

Average number of seeds per silique increased proportionate to silique length ($y = 0.3898x - 9.1182$, $R^2 = 0.65442$, $P < 0.0001$) and average seed weight ($y = 0.00001x - 0.0002$, $R^2 = 0.37297$, $P < 0.0001$). Due to these strong correlations, only the average number of seeds per silique was used for analysis instead of average seed weight or length.

Geographic and Genetic Isolation

Linear Geographic Distance Between Parents

Fitness

Linear geographic distance did not predict average seeds produced per silique (linear regression $y = 1.059x + 18.607$, $R^2 = 0.00584$, $F_{1,116} = 0.682$, $P = 0.411$; Figure 7).

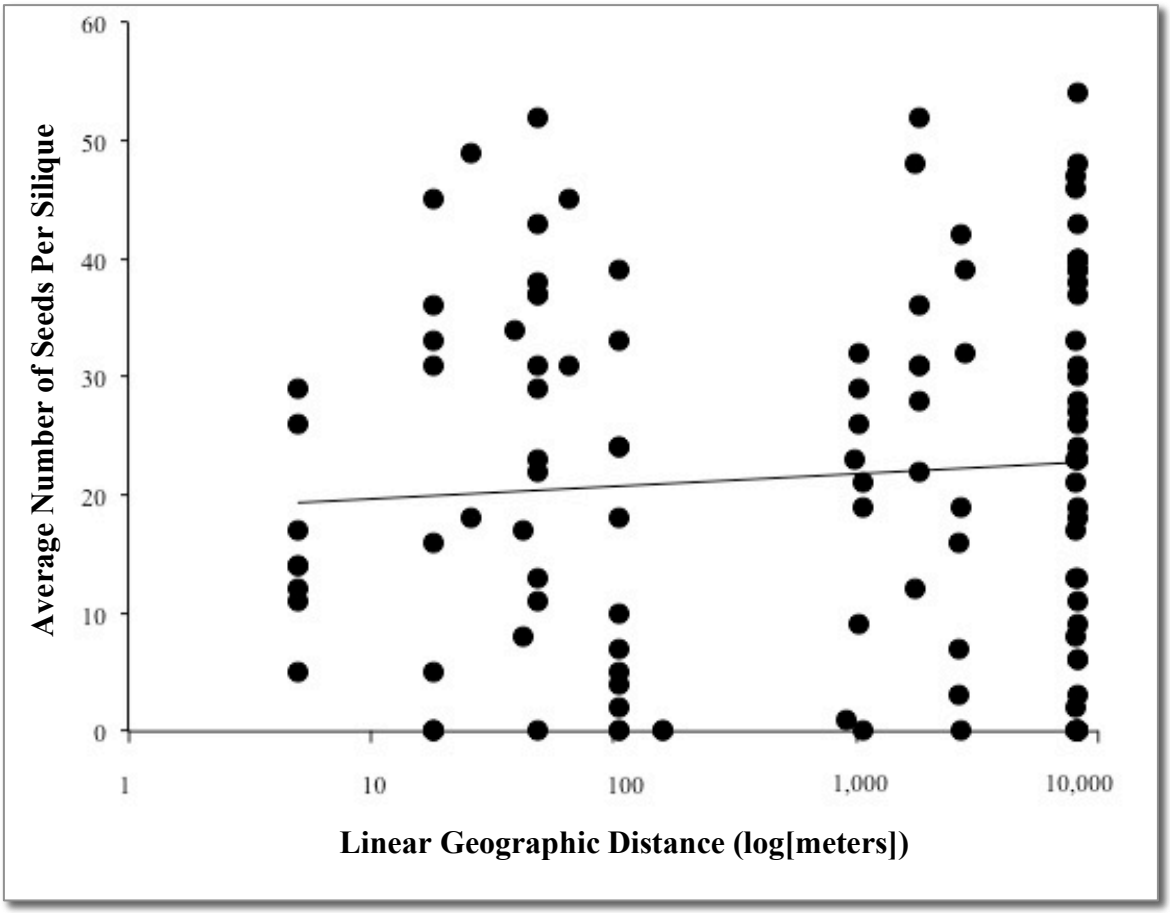

Figure 7. Linear geographic distance vs. fitness. Linear geographic distance is not a predictor for plant fitness. Linear regression $y = 1.059x + 18.607$, $R^2 = 0.00584$, $F_{1,116} = 0.682$, $P = 0.411$.

Genetic Divergence

Linear geographic distance is a weak predictor for genetic divergence between parent crosses because even though increasing linear distance reflects increasing genetic divergence, the R^2 is low (linear regression $y = 0.022x + 0.037$, $R^2 = 0.044$, $F_{1,116} = 5.289$, $P = 0.0232$; Figure 8).

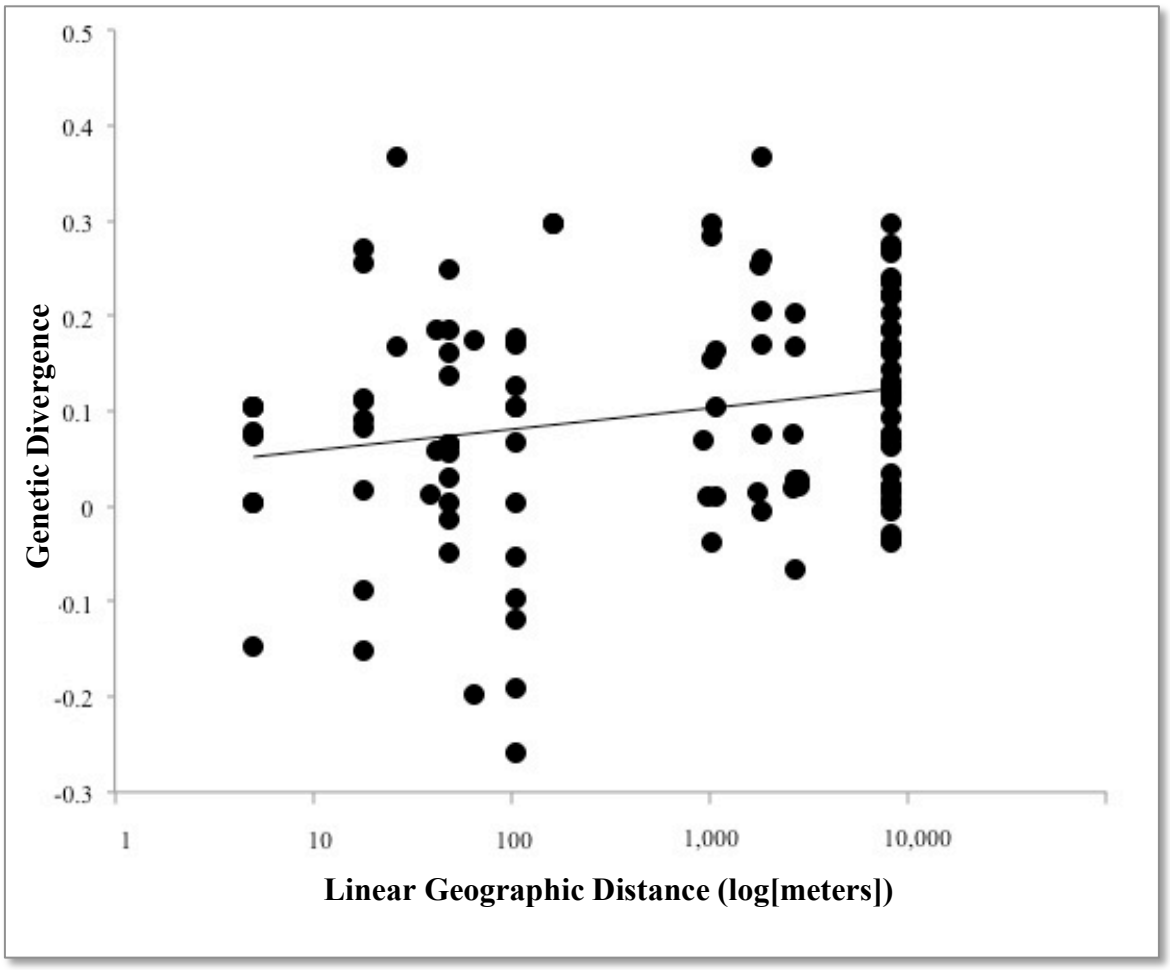

Figure 8. Linear geographic distance vs. genetic divergence. Linear geographic distance is not a predictor for genetic divergence. Linear regression $y = 0.022x + 0.037$, $R^2 = 0.044$, $F_{1,116} = 5.289$, $P = 0.0232$.

Nonlinear Geographic Distance (Habitat Barriers) Between Parents

Overall, seed set increased significantly with nonlinear geographic distance (habitat barriers). As described above, seed set was lowest in self-pollinated plants. In addition, though, within-population seed set was lower than between-population seed set (within-population crosses $n = 50$; between-population crosses $n = 68$; Kruskal Wallis test, $P < 0.0001$; Dwass-Steel-Chritchlow-Fligner $P < 0.0001$ for all crosses; Figure 9).

Within-population crosses (19.96 seeds/silique) produced about 13% fewer seeds than between-population crosses (22.69 seeds/silique).

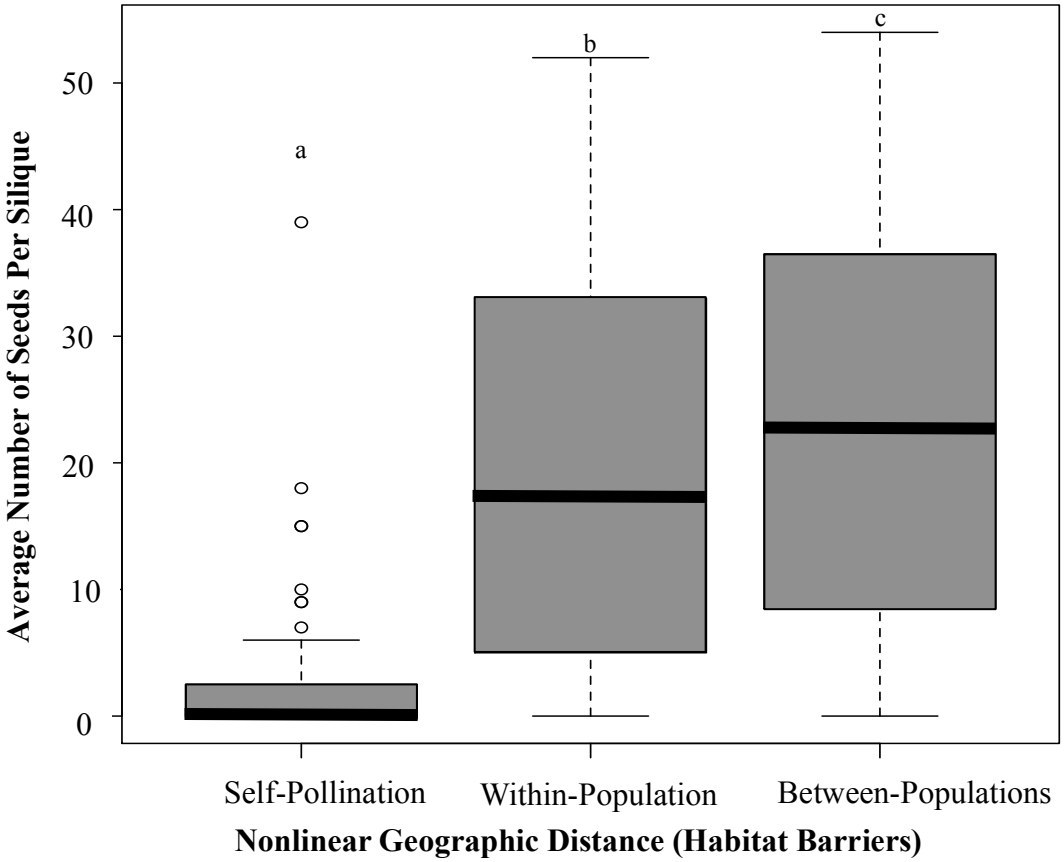

Figure 9. Nonlinear geographic distance (habitat barriers) vs. plant fitness. Within-population crosses produced about 13% fewer seeds than between-population crosses. Lower case letters denote significant differences between medians ($P < 0.05$). A Tukey boxplot showing the median within the interquartile (IQR) range and whiskers of 1.5 IQR. Outliers are represented as open dots.

Characteristics of the crosses that produce the lowest and highest number of seeds were examined by analyzing the frequency distribution of seed set. Slightly fewer crosses produced siliques with zero seeds in the between-population crosses compared to within-population crosses (9/68 = 13.2% vs. 8/50 = 16.0%, respectively). When

examining the top quartile of seed producing crosses among all outcross treatments (n = 30 out of a total of 118), there was an excess of between-population crosses (63%) compared to within-population crosses (37%). Given the unequal sample sizes, the predicted percentage of crosses in the top quartile should be 57.6% from between-population crosses and 42.4% from within-population crosses, if they were equal. Even when accounting for unequal sample sizes, there was still 5% excess of between-population crosses among the top seed-producing crosses. When analyzing the mean seed set in the top quartile, it was not significantly different between the two outcross types (between-population = 42.2 seeds/silique, within-population = 40.7 seeds/silique; t-test, $P > 0.05$). The conclusion was that the differences in seed production in both the lowest and highest quartiles were contributing to the weakly significant difference between the two nonlinear outcrossing categories (within-population and between-population).

Genetic Divergence Between Parents

When all cross data were included (self-pollination and outcross data), seed count significantly increased with genetic divergence (linear regression $y = 40.3x + 16.459$, $R^2 = 0.222$, $F_{1,159} = 45.438$, $P < 0.0001$; Figure 10). However, the mating system experiment demonstrated that self-pollinations produced low seed set in general. Therefore, the analysis was run to only include outcrossed parents, to see if genetic inbreeding might explain the loss of fitness in crosses between nearby parent plants. When self-pollination data are removed, genetic divergence did not explain seed count (n = 118, linear

regression $y = 40.3x + 16.459$, $R^2 = 0.0151$, $P > 0.05$; Figure 11). Therefore, self-pollination genetic data, which have many zero values, are driving the significance of the earlier result (Figure 10).

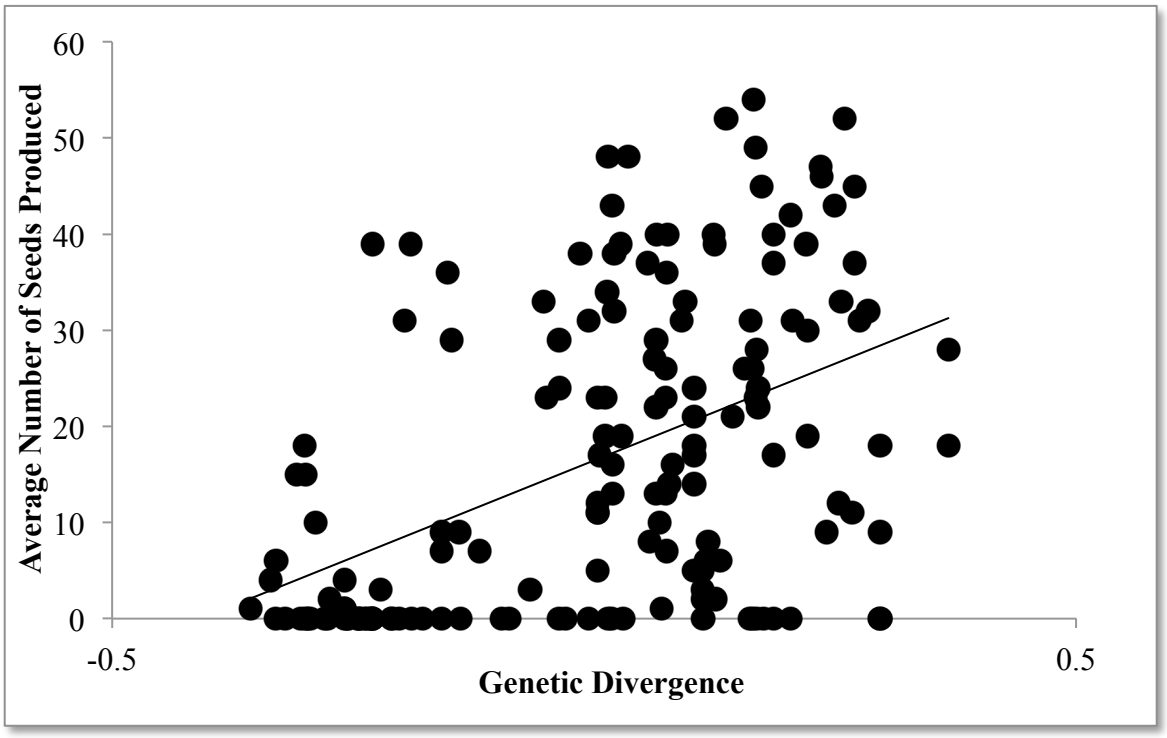

Figure 10. Genetic divergence vs. self-pollination and outcross fitness. Smaller genetic divergence numbers denoted parent combinations that were more genetically similar, whereas larger genetic divergence numbers indicated parent combinations that were more genetically divergent. Linear regression $y = 40.3x + 16.459$, $R^2 = 0.222$, $F_{1,159} = 45.438$, $P < 0.0001$.

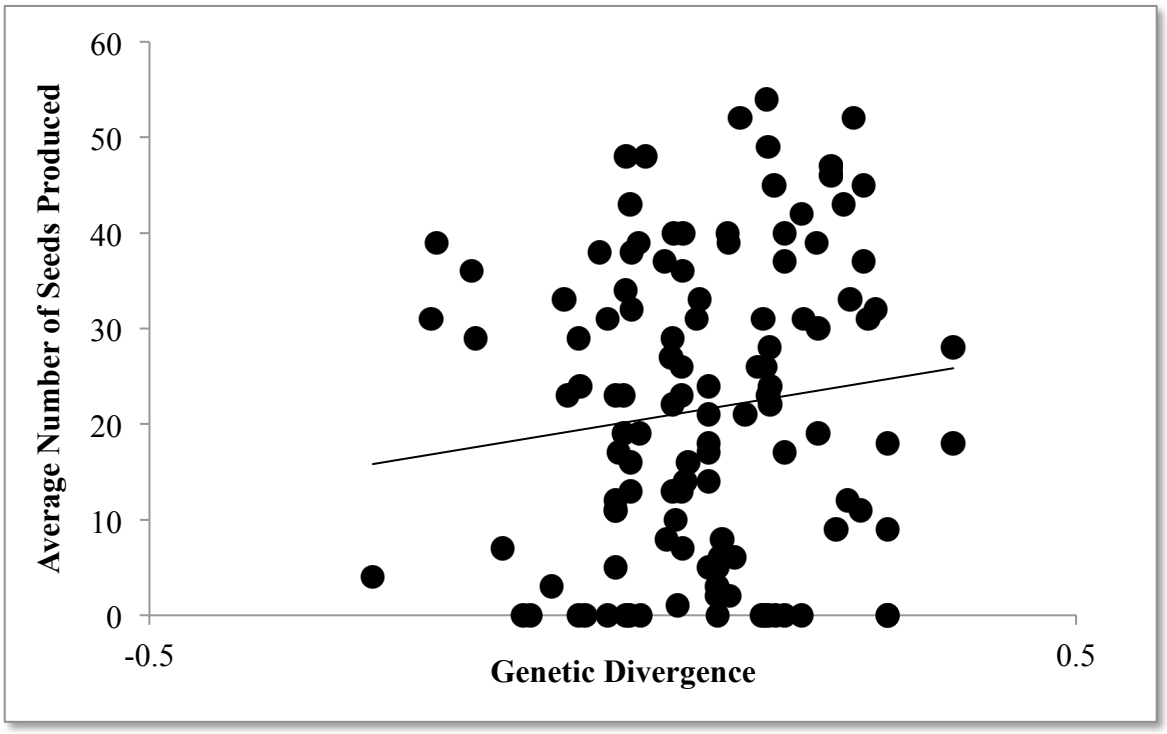

Figure 11. Genetic divergence vs. outcross fitness with self-pollinations removed. Smaller genetic divergence numbers denoted parent combinations that were more genetically similar, whereas larger genetic divergence numbers indicated parent combinations that were more genetically divergent. Linear regression $y = 16.005x + 19.965$, $R^2 = 0.015$, $F_{1,116} = 1.778$, $P = 0.185$.

Population Source

When comparing each source population, outcross seed set was the greatest for Quail Hollow Park and Water District, which were significantly different from Morgan Preserve and Eco Reserve ($n = 118$; Kruskal-Wallis test, $P = 0.002$; Dwass-Steel-Chritchlow-Fligner $P \leq 0.001$ for all crosses; Figure 12).

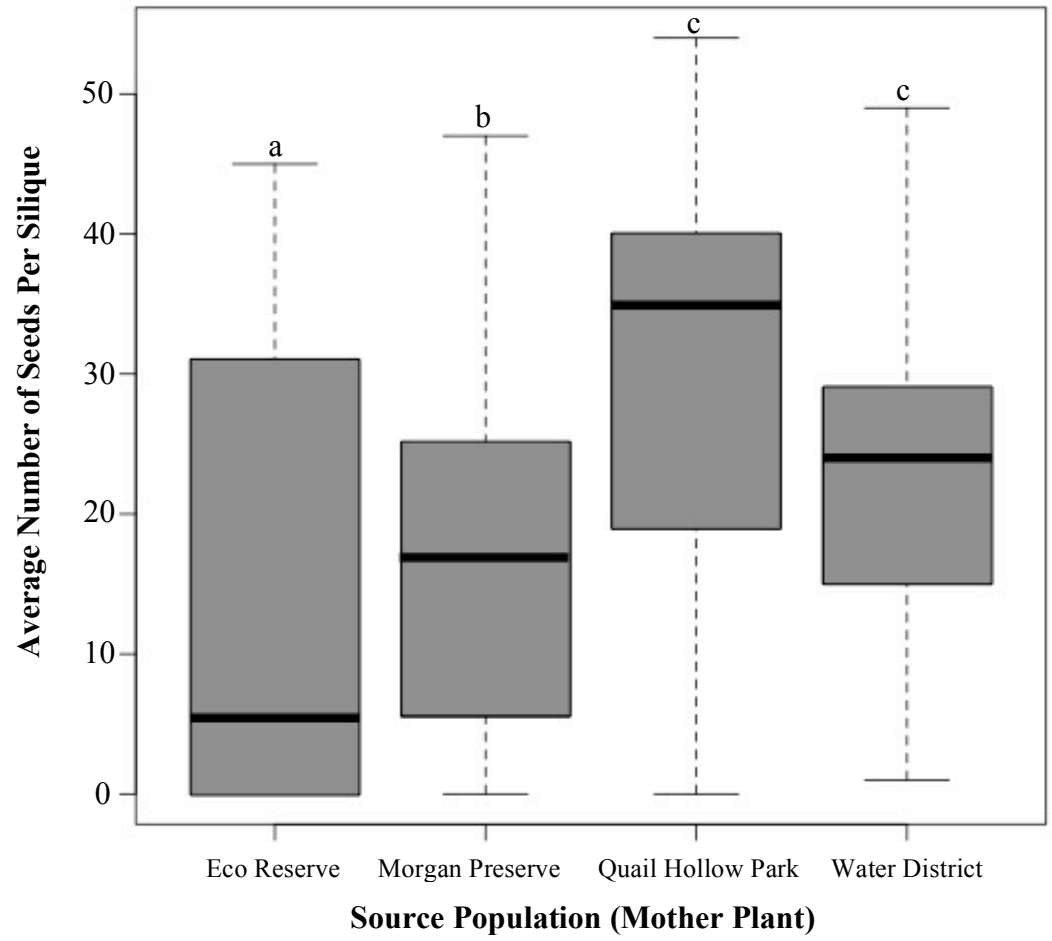

Figure 12. Population source vs. fitness. Outcross seed set was the highest for Quail Hollow Park and Water District, which was significantly different from Morgan Preserve and Eco Reserve (n = 118; Kruskal-Wallis test, P = 0.002). Lower case letters denote significant differences between medians (P < 0.05). A Tukey boxplot showing the median within the interquartile (IQR) range and whiskers of 1.5 IQR. Outliers are represented as open dots.

Pollinator Survey

During the spring survey of pollinators, a diverse array of Hymenoptera (bees and wasps), Lepidoptera (butterflies and moths), and Diptera (flies) were identified while visiting *E. teretifolium*. The insects in the video survey tend to be native and many are

strong fliers. There were 125 visits per flower per hour, which resulted in a rate of 0.827 visits per flower hour.

DISCUSSION

Many species in the Brassicaceae family have sporophytic self-incompatibility systems (Anderson and Busch 2006; Charlesworth et al. 2005) and, thus, are reliant on pollinators (Ortigosa and Gómez 2010; Pavlik, Ferguson, and Nelson 1993). However, in fragmented habitats where mate selection is low, self-compatible mating systems can arise because a single plant can colonize an area (Busch 2005; Pannell and Barrett 1998). When comparing self-pollinations and outcross pollinations for *E. teretifolium*, seed production decreased (6.5 times fewer seeds) with self-pollinations. Due to this dramatic difference in seed set, it is likely that *E. teretifolium* has a self-incompatible mating system and therefore relies on outcrossing for reproduction. The percent of crosses producing zero seeds was 4.4 times higher for self-pollinations (27/43 = 62.8%) compared to outcross pollinations (17/118 = 14.4%).

Island biogeography, designed for oceanic islands, is expected to apply to continental island-like habitats that exhibit patchy distributions (Barbará et al. 2008; Edmands 2007; MacArthur and Wilson 1963; Pickett 1985). Levin (1981) expects geographic distance to correlate with genetic distances among populations. In isolated habitats, local adaptation can lead to outbreeding depression, or anthropogenic fragmentation can impact mate selection and drive a population to develop inbreeding depression. In the Santa Cruz Mountains, the sandhills are naturally isolated by mesic forests and fragmented from anthropogenic activities such as urbanization, recreation, and mining. Therefore, it is expected that the sandhills could host species that are detrimentally impacted by extreme fragmentation. Holycross and Douglas (2007) studied

the impacts of continental habitat fragmentation on the genetics of *Crotalus willardi obscurus* (New Mexican ridge-nosed rattlesnake) and concluded that genetic bottlenecks were a result of natural isolation rather than anthropogenic habitat destruction. Duncan et al. (2004) studied pollination success of the partially self-compatible plant, *Dianella revoluta* (Black-anther Flax-lily) in the Gubbata Nature Reserve, Australia. The study populations were artificially fragmented by human-caused soil disturbance. They found that pollen deposition from Hymenoptera did occur, even in the most isolated locations (400 m from the pollen source); however, the levels of outcrossed pollen found on these stigmas were significantly reduced due to geographic isolation. Pollinator visitation rates were determined to be adequate for the area and did not impact pollination success. Duncan et al. (2004) caution that the plant mating system may dictate overall susceptibility to inbreeding.

Results from the geographic and genetic isolation study did not yield clear evidence that island biogeography or fragmentation is impacting *E. teretifolium*. Linear geographic distance between parents does not predict fitness. While between-population crosses performed better than within-population crosses, linear geographic distance between parents poorly predicted genetic divergence. Possible reasons for this include: genetic divergence among parents may somewhat improve fitness, but not enough to be detected; a resilient genetic structure or historical outcrossing from pollinators may have protected populations of *E. teretifolium* by diversifying the gene pool; and/or there may also be an accumulation of self-incompatibility alleles in the genetic source due to the increased fragmentation.

The spring pollinator survey of wild populations of *E. teretifolium* identified common insect pollinators from the orders of Hymenoptera, Lepidoptera, and Diptera. *Erysimum* taxa are typically pollinator-generalists and attract Hymenoptera, Diptera, Lepidoptera, and Coleoptera (Gómez et al. 2007; Ortigosa and Gómez 2010). Many of the insects identified in the pollinator survey were native and considered strong fliers. Jabis, Ayers, and Allan (2011) studied pollinators of *Abronia alpina* (Ramshaw Meadows sand verbena), an alpine endemic, and found that strong fliers such as butterflies and moths (Lepidoptera) are important pollinators capable of dispersing pollen over long distances. Noctuidae (Lepidoptera) are strong fliers and have been estimated to travel 750 km during a two-year study in Arkansas that examined specimens for pollen from distant sources (Hendrix et al. 1987). In a study on Euglossine bees in a tropical rain forest in Costa Rica, Janzen (1971) found that the bees could forage for pollen up to 23 km away.

Although *E. teretifolium* visitation rates were not unusually high, when accounting for the time each flower is available to pollinators, pollination is assured. In the wild, flowers for *E. teretifolium* stay open a minimum of three days (Parker, Weitz, and Webster 2011). In less than ideal conditions (assuming 50% favorable) due to wind, overcast skies, or fog, a conservative estimate is that each flower is potentially visited 15 times during its lifetime. Previous studies have shown that there does not appear to be pollen limitation from poor pollination rates (Parker, Weitz, and Webster 2011); therefore, this rate is adequate for outcrossing for *E. teretifolium*.

REINTRODUCTION PLANS

This study demonstrates that *E. teretifolium* exhibits a self-incompatible mating system with some exceptions that are provocative, but lacking a discernable pattern. Therefore, outcrossing is necessary to improve fitness and promoting pollinators is critical for *E. teretifolium* reproduction. The mating system experiment found that crosses between parents of greater distance perform as well as local crosses, and crosses from parents between populations perform better than crosses within a population. Crosses with a mother plant from Quail Hollow Park and Water District performed better than all other populations. From analysis of linear geographic isolation, nonlinear geographic distance (habitat barriers), and genetic divergence, it appears that island biogeography is not a good predictor for this sandhills species.

The Eco Reserve population appears to be in dire straits. Between 2006 and 2012 it declined from 77 to six individuals, and mothers from this site demonstrated significantly poorer reproductive success than from other populations in the current study (Parker, Weitz, and Webster 2011). This population is clearly experiencing reproductive failure. Since the mating system experiment has hinted at possible self-incompatibility, the Eco Reserve population may be suffering as a result of encountering high levels of self-pollination due to the small number of reproductive adults. Land managers interested in formulating restoration and reintroduction plans for the sandhills need to consider seed collection options for *E. teretifolium*.

There are generally three choices that managers have when collecting seed: (1) collect seeds from the local watershed, (2) introduce seed from other populations to

reduce the chance of incompatibility, and (3) consciously bring in seed from the most successful population (Bischoff et al. 2006; Lesica and Allendorf 1999; McKay et al. 2005; Vander Mijnsbrugge, Bischoff, and Smith 2010). The first option mitigates possible outbreeding depression by mixing seeds within a local source or watershed, which tends to have similar abiotic conditions that drive local adaptation for species. However, of the known remaining populations, the Eco Reserve is found in a different watershed from the other seven populations that were surveyed during a larger genetic study (Herman and Whittall, forthcoming). Collecting local seed from within the watershed is not a viable solution for this population, especially since there are limited reproductive adults in the wild, and the mating system study demonstrated that self-pollinations produced 6.5 times fewer seeds than outcross pollinations.

Herman and Whittall (forthcoming) found that most of the genetic variation (80%) was harbored within each population. Based on the microsatellite data performed in the larger population survey, the geographically disjunct Eco Reserve population did not represent a genetically distinct grouping (Herman and Whittall, forthcoming). Seed set was significantly higher for Quail Hollow Park and Water District. Therefore, those populations are the best options for conscious seed collection to select for robust populations; however, it is important to conduct further research on possible abiotic factors that could impact seed production on non-home soil. Just as it is important to identify where seeds should be collected, the mating system results dictate the need to conserve pollinators. We have some understanding of how to conserve *E. teretifolium*, but further research is required to better manage this endangered species.

Future Research

The next step for this research is to continue common garden experiments using later life history fitness assessment measures, such as the vegetative biomass and reproduction ability of the F1 generation. The fitness measure used for the mating system study was limited in its ability to detect inbreeding depression. Further tests of the F1 generation will provide additional information about possible inbreeding depression, since inbreeding tends to show up in later stages of a plant's natural history (D. Charlesworth and B. Charlesworth 1987; Edmands 2007). It is also important to solidify the presence of a self-incompatible gene by looking at the physiology of *E. teretifolium*.

The mating system study was conducted in a greenhouse setting in Santa Clara, California, which has different conditions from those found at the study sites where the captive breeding population was initially harvested. Therefore, moving beyond common garden experiments to reciprocal transplants is important to determine if soil type or other abiotic conditions are impacting these populations. Without field experiments, it is challenging to know if there are local factors that contribute to the reproductive success of this species.

Finally, managers need to be aware of the importance of insect pollinators for *E. teretifolium*, since the results of the mating system dictate the reliance on outcrossing for fitness. Further studies should survey important pollinators for *E. teretifolium* throughout its flowering season of March through June (McGraw 2004b). It is also important to identify whether the pollinators are generalists or specialists to determine if there is a

need for conservation status, since specialist pollinators are more at risk of extinction (Bawa 1990; Packer et al. 2005; Steffan-Dewenter et al. 2002; Strickler 1979; Waser et al. 1996). An assessment of pollinator habitat requirements will better allow land managers to protect insect pollinators and provide adequate habitat for the sustainability of strong pollinator guilds in the sandhills.

REFERENCES

Aguilar, Ramiro, Lorena Ashworth, Leonardo Galetto, and Marcelo Adrián Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9, no. 8: 968-980.

Aguilar, Ramiro, Mauricio Quesada, Lorena Ashworth, Yvonne Herrerias-Diego, and Jorge Lobo. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* 17, no. 24: 5177-5188.

Anderson, Ingrid A., and Jeremiah W. Busch. 2006. Relaxed pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). *American Journal of Botany* 93, no. 6: 860-867.

Barbará, T., C. Lexer, G. Martinelli, S. Mayo, M. F. Fay, and M. Heuertz. 2008. Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation, *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* (Bromeliaceae). *Heredity* 101, no. 3: 285-296.

Barbour, Clyde D., and James H. Brown. 1974. Fish species diversity in lakes. *The American Naturalist* 108, no. 962: 473-489.

Barrett, Spencer C. H. 2002. The evolution of plant sexual diversity. *Nature Reviews* 3, no. 4: 274-284.

Barrett, Spencer C. H. 2003. Mating strategies in flowering plants: The outcrossing–selfing paradigm and beyond. *Philosophical Transactions of The Royal Society B: Biological Sciences* 358, no. 1434: 991-1004.

Bauer, Paul J. 1983. Bumblebee pollination relationships on the Beartooth Plateau Tundra of Southern Montana. *American Journal of Botany* 70, no. 1: 134-144.

Bawa, K. S. 1990. Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399-422.

Bischoff, Armin, Beatrice Vonlanthen, Thomas Steinger, and Heinz Müller-Schärer. 2006. Seed provenance matters—effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology* 7, no. 4: 347-359.

Bosch, Maria, and Nickolas M. Waser. 2001. Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia* 126, no. 1: 76-83.

Bradshaw, H. D., and Douglas W. Schemske. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* 426, no. 6963: 176-178.

Broadhurst, Linda M., Andrew Lowe, David J. Coates, Saul A. Cunningham, Maurice McDonald, Peter A. Vesk, and Colin Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1, no. 4: 587-597.

Brown, James H. 1971. Mammals on mountaintops: nonequilibrium insular biogeography. *The American Naturalist* 105, no. 945: 467-478.

Busch, Jeremiah W. 2005. The evolution of self-compatibility in geographically peripheral populations of *Leavenworthia alabamica* (Brassicaceae). *American Journal of Botany* 92, no. 9: 1503-1512.

Calsbeek, Ryan, John N. Thompson, and James E. Richardson. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology* 12, no. 4: 1021-1029.

Campbell, Lesley G., and Brian C. Husband. 2007. Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenozys herbacea* (Asteraceae). *New Phytologist* 174: 915-925.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237-268.

Charlesworth, Deborah, Xavier Vekemans, Vincent Castric, and Sylvain Glémin. 2005. Plant self-incompatibility systems: a molecular evolutionary perspective. *New Phytologist* 168, no. 1: 61-69.

Chittka, L., and S. Schürkens. 2001. Successful invasion of a floral market. *Nature* 411, no. 6838: 653.

Chu, Jennifer B. 2002. Diet for an endangered insect: what does the zayante band-winged grasshopper eat? Master's thesis, San José State University.

Cresswell, J. E., A. P. Bassom, S. A. Bell, S. J. Collins, and T. B. Kelly. 1995. Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: A comparison of three models. *Functional Ecology* 9, no. 6: 829-841.

Davis, Edward Byrd, Michelle S. Koo, Chris Conroy, James L. Patton, and Craig Moritz. 2007. The California hotspots projects: identifying regions of rapid diversification of mammals. *Molecular Ecology* 17, no. 1: 120-138.

Dias, Murilo S., Jean François Cornu, Thierry Oberdorff, Carlos A. Lasso, and Pablo A. Tedesco. 2013. Natural fragmentation in river networks as a driver of speciation for freshwater fishes. *Ecography* 36, no. 6: 683-689.

Duncan, David H., Adrienne B. Nicotra, Jeff T. Wood, and Soul A. Cunningham. 2004. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. *Journal of Ecology* 92, no. 6: 977-985.

Eckert, Christopher G., and Amy Schaefer. 1998. Does self-pollination provide reproductive assurance in *Aguilegia canadensis* (Ranunculaceae)? *American Journal of Botany* 85, no. 7: 919-924.

Eckert, Christopher G., Susan Kalisz, Monica A. Geber, Risa Sargent, Elizabeth Elle, Pierre-Olivier Cheptou, Carol Goodwillie, Mark O. Johnston, John K. Kelly, David A. Moeller, Emmanuelle Porcher, Richard H. Ree, Mario Vallejo-Marín, and Alice A. Winn. 2009. Plant mating systems in a changing world. *Trends in Ecology and Evolution* 25, no. 1: 35-43.

Edmands, Suzanne. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* 16, no. 3: 463-475.

Fenster, Charles B., W. Scott Armbruster, Paul Wilson, Michele R. Dudash, and James D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375-403.

Galen, C, and M. E. A. Newport. 1987. Bumble bee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. *Oecologia* 74, no. 1: 20-23.

Gómez, José M., Jordi Bosch, Francisco Perfectti, Juande Fernández, and Mohamed Abdelaziz. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* 153, no. 3: 597-605.

Goodwillie, Carol, Susan Kalisz, and Christopher G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* 47-79.

Google Earth™. 2013. Version 7.1.1.1888. Mountain View: Google, Inc.

Granite Rock Company. 1998. *Revised habitat conservation plan.* Watsonwille, CA: Granite Rock Company.

Grashof-Bokdam, C. J., W. Geertsema. 1998. The effect of isolation and history on colonization patterns of plant species in secondary woodland. *Journal of Biogeography* 25: 837-846.

Guo, Ya-Long, Jesper S. Bechsgaard, Tanja Slotte, Barbara Neuffer, Martin Lascoux, Detlef Weigel, and Mikkel H. Schierup. 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the United States of America* 106, no. 13: 5246-5251.

Hendrix, W. H., T. F. Mueller, J. R. Phillips, and O. K. Davis. 1987. Pollen as an indicator of long-distance movement of *Heliothis zea* (Lepidoptera: Noctuidae). *Environmental Entomology* 16, no. 5: 1148-1141.

Herman, Julie, and Justen Whittall. Unexpected levels of gene flow in a wallflower occupying an island-like habitat (*Erysimum teretifolium,* Brassicaceae). Forthcoming *Conservation Genetics*.

Hill, Kirsten E., and Rachel O'Malley. 2010. A picky palate? The host plant selection of an endangered June beetle. *Journal of Insect Conservation* 14, no. 3: 277-287.

Holycross, Andrew T., Michael E. Douglas. 2007. Geographic isolation, genetic divergence, and ecological non-exchangeability define ESUs in a threatened sky-island rattlesnake. *Biological Conservation* 134, no. 1: 142-154.

Honnay, Olivier, and Hans Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21, no. 3: 823-831.

Jabis, Meredith D., Tina J. Ayers, and Gerard J. Allan. 2011. Pollinator-mediated gene flow fosters genetic variability in a narrow alpine endemic, *Abronia alpina* (Nyctaginaceae). *American Journal of Botany* 98, no. 10: 1583-1594.

Jablonski, David, and W. G. Chaloner. 1994. Extinctions in the fossil record [and discussion]. *Philosophical Transactions: Biological Sciences* 344, no. 1307: 11-17.

Janzen, D. H. 1971. Euglossine bees as long-distance pollinators of tropical plants. *Science* 171, no. 3967: 203-205.

Jepson Flora Project, eds. 2013. *Jepson eFlora*, http://ucjeps.berkeley.edu/IJM.html, [accessed March 18, 2014].

Kao, Teh-hui, and Andrew G. McCubbin. 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proceedings of the Natural Academy of Sciences of the United States of America* 93: 12059-12065.

Kremen, Claire, Robert L. Bugg, Nikki Nicola, Sarah A. Smith, Robbin W. Thorp, and Neal M. Williams. 2002. Native bees, native plants and crop pollination in California. *Fremontia* 30, no. 3-4: 41-49.

Kremen, Claire, Neal M. Williams, Robert L. Bugg, John P. Fay, and Robbin W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* 7, no 11: 1109-1119.

Lesica, Peter, and Fred W. Allendorf. 1999. Ecological genetics and the restoration of plant communities: mix or match? *Restoration Ecology* 7, no. 1: 42-50.

Levin, Donald A. 1981. Dispersal versus gene flow in plants. *Annals of the Missouri Botanical Garden* 68, no. 2: 233-253.

Levin, Donald A. 1984. Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. *Evolution* 38, no. 1: 116-127.

MacArthur, Robert H., and Edward O. Wilson. 1963. An equilibrium theory of insular zoogeography. *Evolution* 17, no. 4: 373-387.

Mayer, Carolin, Fabienne Van Rossum, and Anne-Laure Jacquemart. 2012. Evaluating pollen flow indicators for an insect-pollinated plant species. *Basic and Applied Ecology* 13, no. 8: 690-697.

McGraw, Jodi M., and Anna L. Levin. 1998. The roles of soil type and shade intolerance in limiting the distribution of the edaphic endemic *Chorizanthe Pungeus* var. *Hartwegiana* (Polygoaceae). *Madroño* 45, no. 2: 119-127.

McGraw, Jodi M. 2004a. The sandhills conservation and management plan: a strategy for preserving native biodiversity in the Santa Cruz sandhills. A report submitted to the Land Trust of Santa Cruz County, Santa Cruz, CA. http://www.santacruzsandhills.com/articles/Sandhills%20Conservation%20and%20Management%20Plan%20June%202004.pdf.

McGraw, Jodi Marie. 2004b. Interactive effects of disturbance and exotic species on the structure and dynamics of an endemic sandhills plant community. PhD diss., University of California, Berkeley.

McKay, John K., Caroline E. Christian, Susan Harrison, and Kevin J. Rice. 2005. "How local is local?" – a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13, no. 3: 432-440.

Mena-Ali, Jorge I., Lidewij H. Keser, and Andrew G. Stephenson. 2008. Inbreeding depression in *Solanum carolinense* (Solanaceae) species with a plastic self-incompatibility response. *BMC Evolutionary Biology* 8, no. 10: 1-10.

Muñoz-Pajares, A. Jesús, M. Belén Herrador, Mohamed Abdelaziz, F. Xavier Picó, Timothy F. Sharbel, José M. Gómez, and Francisco Perfectti. 2011. Characterization of microsatellite loci in *Erysimum mediohispanicum* (Brassicaceae) and cross-amplification in related species. *American Journal of Botany* 98, no. 10: e287-e289.

Murphy, Kathryn Ann. 2003. Endangered plant conservation: do pollinators matter to the robust spineflower? Master's thesis, San José State University.

Ortigosa, Angel L., and José M. Gómez. 2010. Differences in the diversity and composition of the pollinator assemblage of two co-flowering congeneric alpine wallflowers, *Erysimum nevadense* and *E. baeticum*. *Flora* 205, no. 4: 266-275.

Packer, Laurence, Amro Zayed, Jennifer C. Grixti, Luisa Ruz, Robin E. Owen, Felipe Vivallo, and Haroldo Toro. 2005. Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. *Conservation Biology* 19, no. 1: 195-202.

Pannell, John R., and Spencer C. H. Barrett. 1998. Baker's law revisited: reproductive assurance in a metapopulation. *Evolution* 52, no. 3: 657-668.

Parker, Ingrid M., Andrew Weitz, and Kelsey Webster. 2011. Reproductive bottlenecks in Ben Lomond Wallflower (*Erysimum teretifolium*). University of California, Santa Cruz.

Pavlik, Bruce M., Nancy Ferguson, and Marjorie Nelson. 1993. Assessing limitations on the growth of endangered plant populations, II. Seed production and seed bank dynamics of *Erysimum capitatum* ssp. *Angustatum* and *Oenothera deltoids* ssp. *Howellii*. *Biological Conservation* 65, no. 3: 267-278.

Pickett, Steward T. 1985. *The ecology of natural disturbance and patch dynamics*. Orlando, FL: Academic Press, Inc.

Potts, Simon G., Betsy Vulliamy, Amots Dafni, Gidi Ne'eman, and Pat Willmer. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology* 84, no. 10: 2628-2642.

Price, Mary V., Nickolas M. Waser, Rebecca E. Irwin, Diane R. Campbell, and Alison K. Brody. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology* 86, no. 8: 2106-2116.

Price, Robert Allen. 1987. Systematics of the *Erysimum capitatum* alliance (Brassicaceae) in North America. PhD diss., University of California, Berkeley.

Quinn, James F., and Susan P. Harrison. 1988. Effects of habitat fragmentation and isolation on species richness: evidence from biogeographic patterns. *Oecologia* 75, no. 1: 132-140.

Raup, David M., and J. John Sepkoski. 1982. Mass extinctions in the marine fossil record. *Science* 215, no. 4539: 1501-1503.

Rice, Kevin J., and Nancy Emery. 2003. Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment* 1, no. 9: 469-478.

Richter, Stephen C., Brian I. Crother, and Richard E. Broughton. 2009. Genetic consequences of population reduction and geographic isolation in the critically endangered frog, *Rana sevosa*. *Copeia*, no. 4: 801-808.

Saunders, Denis A., Richard J. Hobbs, and Chris R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5, no. 1: 18-32.

Scott, Thomas, Richard Standiford, and Nanette Pratini. 1995. Private landowners critical to saving California biodiversity. *California Agriculture* 49, no. 6: 50-57

Steets, Janette A., Diana E. Wolf, Josh R. Auld, and Tia-Lynn Ashman. 2007. The role of natural enemies in the expression and evolution of mixed mating in hermaphroditic plants and animals. *Evolution* 61, no. 9: 2043-2055.

Steffan-Dewenter, Ingolf, Ute Münzenberg, Christof Bürger, Carsten Thies, and Teha Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83, no. 5: 1421-1432.

Steffen, Will, Paul J. Crutzen, and John R. McNeill. 2007. The Anthropocene: are humans now overwhelming the great forces of nature? *Ambio* 36, no. 8: 614-621.

Strickler, Karen. 1979. Specialization and foraging efficiency of solitary bees. *Ecology* 60, no. 5: 998-1009.

U.S. Department of Agriculture. 1980. *Soil survey of Santa Cruz County, California*. Soil conservation service in cooperation with University of California, Agricultural Experiment Station. U.S. Government Printing Office.

U.S. Fish and Wildlife Service. 1997. Draft recovery plan for two insects (*Polyphylla barbata* and *Trimerotropis infantilis*) and four plants (*Chorizanthe pungens* var. *hartwegiana*, *Chorizanthe robusta* var. *hartwegii*, *Erysimum teretifolium*, and *Polygonum hickmanii*) from the Santa Cruz Mountains, California. Portland, Oregon.

U.S. Fish and Wildlife Service. 1998. Recovery plan for insect and plant taxa from the Santa Cruz Mountains in California, by Connie Rutherford and Kim Touneh. Portland, Oregon.

Vander Mijnsbrugge, Kristine, Armin Bischoff, and Barbara Smith. 2010. A question of origin: where and how to collect seed for ecological restoration. *Basic and Applied Ecology* 11, no. 4: 300-311.

Wake, David B., and Vance T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the Natural Academy of Sciences of the United States of America* 105, no. Supplement 1: 11466-11473.

Waser, Nickolas M, Lars Chittka, Mary C. Price, Neal M. Williams, and Jeff Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77, no. 4: 1043-1060.

Welzel, Kevin. 2011. Pollination by native bee communities in Berkeley, California. *Berkeley Scientific Journal* 14, no. 2: 1-6.

Willi, Yvonne, Josh Van Buskirk, and Ary A. Hoffmann. 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* 37: 433-458.

Winfree, Rachael, Neal M. Williams, Jonathan Dushoff, and Claire Kremen. 2007. Native bees provide insurance against ongoing honey bee losses. *Ecology Letters* 10, no. 11: 1105-1113.

Wright, Jessica Wilcox, Maureen L. Stanton, and Rosa Scherson. 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* 8, no. 1: 1-21.

Wright, Stephen I., Susan Kalisz, and Tanja Slotte. 2013. Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society B: Biological Sciences* 280, no. 1760: 1-10.

Young, Helen J., Dan W. Dunning, and Kyle W. von Hasseln. 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* 94, no. 7: 1267-1271.